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Effects of mycotoxins, kojic acid and oxalic acid, on biological fitness of *Lygus hesperus* (Heteroptera: Miridae)

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Mycotoxins kojic acid and oxalic acid are produced by many species of fungi including *Aspergillus niger*, a common contaminant in insectaries. Many species of fungi, including *Aspergillus* spp., have been reported to produce mycotoxins that are toxic to insects (Tanada and Kaya, 1993). *A. flavus* var. *columnaris* produced a water-soluble compound, later shown to be kojic acid, toxic to immature milkweed bugs, *Oncopeltus fasciatus* (Dallas), house flies, *Musca domestica* Linnaeus, and a mosquito, *Aedes atropalpus* (Beard and Walton, 1969). The authors found that kojic acid was low in acute toxicity, but it acted to retard insect development and could be insecticidal in high concentrations, 0.15% and above in drinking water or soaked with milkweed seed, dried, and given as food. *A. niger* can produce a variety of mycotoxins including oxalic acid, kojic acid, and cyclic pentapeptides called malformins (Kobbe et al., 1977; Wilson, 1966). *A. niger* (USDA, ARS Robert T. Gast Rearing Laboratory, Mississippi State, MS isolate) has been previously shown by this laboratory to have detrimental effects on the biological fitness of *Lygus hesperus* Knight (Heteroptera: Miridae) (Alverson, 2002). The purpose of the present study was to test the hypothesis that kojic acid and oxalic acid are partially responsible for the toxic effects of *A. niger* on *L. hesperus.* The mycotoxins were incorporated into an artificial diet used to rear the insect, and the effects on biological fitness of *L. hesperus* were measured. Components defining biological fitness included total number of surviving adults, mean biomass (dry weight) accumulated per cage over the total test period, egg production, number of days to adult emergence, and number of days to the beginning of egg laying. Kojic acid and oxalic acid were found to have detrimental effects on all of the components of biological fitness, suggesting that the mycotoxins may be partially responsible for the toxic effects of *A. niger* on *L. hesperus.*

Lygus hesperus used in these studies were derived from a colony from Biotactics (Riverside, CA), reared on the “C diet” at the Robert T. Gast Rearing Laboratory for 1.5 years (Cohen, unpub. data), and then on the new NI diet (Cohen, 2000) for 41 generations (as of January 2002) prior to the present study. *L. hesperus* adults for this study were placed in the Mississippi State University Entomology Museum as voucher specimens.

Experimental set-up, treatment of insects, and data collection for measurements of biological fitness were as previously described (Alverson and Cohen, 2002). Kojic acid (2-hydroxymethyl-5-hydroxy-γ-pyrone) and oxalic acid (ethanedioic acid) were purchased from Sigma (St. Louis, MO). The diet consisted of NI diet prepared as previously described (Cohen, 2000) except the formalin and propionic acid were omitted. All diet preparations were carried out in a biological safety cabinet (Nuaire, model NU-301-430, Plymouth, MN). Warm diet (20 ml at approximately 45 °C) was dispensed into parafilm feeding packets as previously described (Alverson and Cohen, 2002). Chemical treatments were as follows: control diet, no additive; three concentrations of kojic acid (500, 1000, and 1500 ppm); and three concentrations of oxalic acid (500, 1000, and 1500 ppm). These concentrations were chosen based on previous studies of mycotoxin production by *Aspergillus* spp. (Ogawa et al., 1995; Wei et al., 1991), and were considered to be within a range likely to occur when insect diets are contaminated by *A. niger* (Singh and Bucher, 1971). The tops of the feeding packets were then sealed with a heat sealer. Sterile water was the solvent for the acids. The pH of the test diets was 5.5 ± 0.2.

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A randomized complete block design with seven treatments was used. Treatments were kojic and oxalic acids tested at three concentrations each, plus a control with no toxin added. Four replications were performed over time.

To begin all tests, rearing units consisting of individual cages, described previously (Alverson and Cohen, 2002), were set up and a Parafilm packet containing 200 eggs was placed in each cage. All test eggs for each replication were collected within a 2-h oviposition period in order to synchronize egg-hatch as much as possible. Egg eclosure for the experimental egg packets was complete within a 6-h time frame. Hatch rates from the original egg packs used to begin the experiment and from the F1 egg packs were calculated based on counts of microscopically observed opened opercula and empty eggs. Hatch rates were 90–95% for all cages (data not shown). Cages were examined daily and the following biological parameters were measured for each treatment group: (1) total number of surviving adults; (2) the insect biomass (dry weight) accumulated per cage over the total treatment period, including adults, deceased nymphs, and exuviae; (3) number of days to first adult emergence; (4) number of days to beginning of egg laying; and (5) number of eggs produced by each cage of adults per day for five days of peak egg laying after all survivors had emerged into adults (number of eggs produced per female per day calculated from number of surviving females). Eggs were counted by microscopic examination while still in the gel packets. The experiment was terminated 26 days after nymphal eclosure from eggs (Debolt and Patana, 1985). Surviving adults were collected, sexed, and killed by freezing at −20 °C for 4–6 h. Dead nymphs and exuviae were also collected from the cages at the termination of the experiment and frozen. Dry weights were obtained after drying both collections at 70 °C for 48 h. The collections from each cage were combined to determine total biomass. Data were analyzed using regression analysis to assess the effect of acid concentration on biological fitness and to compare the kojic acid and oxalic acid treatments (SAS Institute, 2000).

The effects of increasing concentrations of kojic and oxalic acid on biological fitness of *L. hesperus* are presented in Table 1. Regression analysis showed that all of the characteristics of biological fitness were negatively affected by increasing doses of kojic and oxalic acid (Table 2). There were not, however, always significant

Table 1
Effect of kojic acid and oxalic acid in NI artificial diet on biological fitness of *L. hesperus*

Mycotoxin	Concentration (ppm)	Total number of surviving adults	Total biomass (dry weight in mg)	Eggs per female per day	Days to adult emergence	Days to oviposition
Control	—	188.0 ± 3.7	921.6 ± 15.0	24.8 ± 0.4	17.0 ± 0	19.0 ± 0
Kojic acid	500	176.3 ± 7.0	858.3 ± 44.5	16.3 ± 0.6	17.0 ± 0	20.0 ± 0
	1000	145.5 ± 11.3	708.7 ± 40.4	8.0 ± 2.8	17.5 ± 0.3	20.5 ± 0.3
	1500	154.0 ± 9.4	655.2 ± 36.4	5.0 ± 1.2	17.5 ± 0.3	20.8 ± 0.3
Oxalic acid	500	167.8 ± 4.5	721.1 ± 58.7	14.4 ± 1.1	17.3 ± 0.3	20.0 ± 0
	1000	158.3 ± 8.2	695.3 ± 92.9	11.4 ± 1.5	17.5 ± 0.3	20.5 ± 0.3
	1500	156.0 ± 11.4	694.5 ± 24.1	8.0 ± 0.7	17.5 ± 0.3	20.8 ± 0.3

Table 2
Regression analysis of effect of kojic acid (ka) and oxalic acid (oa) on biological fitness of *L. hesperus*

Measurement of biological fitness	Slope	Standard error of slope	Null Hypothesis (H_0:)	F value	Pr > F
Total biomass	−0.162	0.041	H_0:ka slope = 0	15.445	0.01
	−0.169	0.041	H_0:oa slope = 0	16.728	0.01
			H_0:slope ka = oa	0.300	0.88
Total number of surviving adults	−4.565[a]	1.224	H_0:ka slope = 0	13.913	0.01
	−4.209[a]	1.224	H_0:oa slope = 0	11.834	0.02
			H_0:slope ka = oa	0.102	0.76
Eggs per female per day	−0.001[a]	0.0001	H_0:ka slope = 0	124.546	0.0001
	−0.0001[a]	0.0001	H_0:oa slope = 0	59.136	0.0001
			H_0:slope ka = oa	11.492	0.002
Days to adult emergence	0.0004	0.0001	H_0:ka slope = 0	9.797	0.004
	0.0004	0.0001	H_0:oa slope = 0	12.041	0.002
			H_0:slope ka = oa	0.096	0.761
Days to oviposition	0.0012	0.0002	H_0:ka slope = 0	42.380	0.001
	0.0012	0.0002	H_0:oa slope = 0	42.380	0.001
			H_0:slope ka = oa	0	1.000

[a] Slope based on log scale. Regression analysis, SAS Institute, software version 8.01, 2000.

differences between the two different acids. The number of eggs per female per day was the only measurement with a significant difference in the rate of decline (slope) between the two different acids with kojic acid having a more detrimental effect on egg production than oxalic acid. Few adults died in the tests, most insect death occurred in the nymphal stage for all diet treatments. The eggs produced by each treatment hatched at a rate comparable to the parent *L. hesperus* colony (90–95%, data not shown).

The effects on biological fitness by the acids, at the concentrations tested, were similar in several respects to the effects shown by *A. niger* (USDA, ARS Robert T. Gast Rearing Laboratory, Mississippi State, MS isolate) inoculated diet (Alverson, 2002). *L. hesperus* reared on diet with experimental inoculation of *A. niger* also showed increased nymphal mortality (29%) when compared to a control. Mean biomass was decreased by 29% in insects reared on diet experimentally inoculated with *A. niger* compared to insects reared on a control diet with no *A. niger* inoculation, similar to the present results from treatment diets with higher concentrations of toxins (23% for 1000 ppm kojic acid, 25% for both 1000 and 1500 ppm oxalic acids, and 29% for 1500 ppm kojic acid). Egg production was decreased by 77% in insects reared on diet experimentally inoculated with *A. niger* compared to the control. This is similar to the results from the treatment diets with the highest concentration of kojic acid (80% decrease in egg production). The time to adult emergence was significantly delayed in insects reared on diets experimentally inoculated with *A. niger* (2.4 days), while it was delayed by 0.5 days in insects reared on diets with high concentrations of kojic acid or oxalic acid incorporated into them. The time to start of egg laying was delayed by 2.6 days in insects reared on diets experimentally inoculated with *A. niger,* and was delayed by 1.0–1.8 days in the present study.

As stated previously, the concentrations of kojic acid and oxalic acid used in the present study were based on previous studies of mycotoxin production by *Aspergillus* spp. (Ogawa et al., 1995; Wei et al., 1991), and concentrations thought likely to occur with *A. niger* contamination in the diet (Singh and Bucher, 1971). A quantitative analysis of kojic acid and oxalic acid production by *A. niger* (Robert T. Gast Rearing Laboratory isolate) would be required to determine the precise concentration of these acids delivered to diet experimentally inoculated with the isolate. However, the results of the present study suggest that the toxic properties of *A. niger* on *L. hesperus* may be caused in part by kojic and/or oxalic acid, confirming the original hypothesis. The highest concentrations of kojic and oxalic acids produced effects on total number of survivors, total biomass, and egg production comparable to those seen with an experimental inoculation of diet with *A. niger*. However, the development time for the insects to emerge as adults was not as severely delayed in the mycotoxin study suggesting that an additional factor(s) may be present in the *A. niger* inoculum to account for this toxic effect. It is also possible that there is a combined synergistic or additive effect when the two mycotoxins are present together as may occur in *A. niger* diet inoculation experiments. *A. niger,* and hence, kojic acid and oxalic acid, may contribute to some extent to the chronic decline of laboratory-reared *L. hesperus* colonies by reducing egg production and causing increased nymphal mortality.

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References

Alverson, J., 2002. Effects of *Aspergillus niger* contamination on biological fitness of *Lygus hesperus* (Heteroptera: Miridae). J. Entomol. Sci. 37, 338–344.

Alverson, J., Cohen, A.C., 2002. Effect of antifungal agents on biological fitness of *Lygus hesperus* Knight (Heteroptera: Miridae). J. Econ. Entomol. 95, 256–260.

Beard, R.L., Walton, G.S., 1969. Kojic acid as an insecticidal mycotoxin. J. Invertebr. Pathol. 14, 53–59.

Cohen, A.C., 2000. New oligidic production diet for *Lygus hesperus* Knight and *L. lineolaris* Palisot de Beauvois. J. Entomol. Sci. 35, 301–310.

Debolt, J.W., Patana, R., 1985. *Lygus hesperus*. In: Singh, P., Moore, R.F. (Eds.), Handbook of Insect Rearing. Elsevier Science, Amsterdam, pp. 329–338.

Kobbe, B., Cushman, M., Wogan, G.N., Demain, A.L., 1977. Production and antibacterial activity of Malformin C, a toxic metabolite of *Aspergillus niger*. Appl. Environ. Microbiol. 33, 996–997.

Ogawa, A., Morita, Y., Tanaka, T., Sakiyama, T., Nakanishi, K., 1995. Production of kojic acid from *Aspergillus oryzae* var. *oryzae* by membrane-surface culture. Biotechnol. Tech. 9, 153–156.

SAS Institute, I. 2000. The SAS system for Windows. 8.01. Cary, NC.

Singh, P., Bucher, G.E., 1971. Efficacy of "safe" levels of antimicrobial food additives to control microbial contaminants in a synthetic diet for *Agria affinas* larvae. Entomol. Exp. Appl. 14, 297–309.

Tanada, Y., Kaya, H.K., 1993. Insect Pathology. Academic Press, San Diego.

Wei, C.I., Huang, T.S., Chen, J.S., Marshall, M.R., Chung, K.T., 1991. Production of kojic acid by *Aspergillus candidus* in three culture media. J. Food Prot. 54, 546–548.

Wilson, B.J., 1966. Toxins produced by *Aspergillus flavus*. Bacteriol. Rev. 30, 460–470.